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Bacterial infection of eggs

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1.1 Introduction

Although a variety of microbes, including pathogens such as *Campylobacter jejuni*, *Listeria monocytogenes* and *Yersinia enterocolitica,* are occasionally found on egg shells and are capable of surviving or growing in egg contents (Burley and Vadehra, 1989; Board and Fuller, 1994; Stadelman and Cotterill, 1995; Ricke *et al.*, 2001), the history of eggs as a source of human illness has almost exclusively concerned bacteria of the genus *Salmonella*. Until the late 1960s, human salmonellosis (involving a diversity of serotypes) was commonly attributed to table eggs with cracked or dirty shells or to egg products that had not been heated sufficiently during processing to completely destroy pathogens. In the USA, the 1970 Egg Products Inspection Act prohibited the sale of cracked and dirty table eggs and mandated reliably effective pasteurization standards for liquid egg products, thereby leading to a very dramatic reduction in the frequency with which human illness was linked to eggs in the years that followed. However, by the mid-1980s, a newly-emerging public health issue again focused attention on eggs as a source of *Salmonella* transmission (St Louis *et al.*, 1988). In this, more recent version of the story of eggs and *Salmonella*, human illness was associated primarily with clean and intact, Grade A table eggs. Moreover, the vast majority of these disease outbreaks involved a single serotype, *S. enterica* serovar Enteritidis (*S.* Enteritidis). An international surge in human *S.* Enteritidis (SE) infections has been principally connected to contaminated eggs (Angulo and Swerdlow, 1999; van de Giessen *et al.*, 1999; Wall and Ward, 1999). In the USA, approximately 80% of the human SE outbreaks for which a food source could be identified have been attributed to eggs or egg-containing foods (Patrick *et al.*, 2004). Accordingly, developing and

implementing effective programmes to diminish the likelihood that consumers will be exposed to contaminated eggs has become an important objective for both government and industry on several continents (Hogue *et al.*, 1997b; Cogan and Humphrey, 2003).

This chapter will explore the causes, characteristics, consequences and control of SE contamination of commercially-produced eggs. Section 1.2 discusses the routes of SE transmission into poultry flocks and into eggs, including the host and bacterial factors that promote the infection of laying hens and how these infections bring about *Salmonella* deposition inside eggs. Section 1.3 discusses the nature of SE contamination of eggs, including the deposition, survival and multiplication of the pathogen in various locations inside eggs. Section 1.4 discusses and assesses the most promising approaches to achieving sustainable, long-term reductions in egg-associated human illness.

1.2 Routes of transmission of *Salmonella* into poultry flocks and eggs

1.2.1 External and internal contamination of eggs

Although extensive microbial contamination of egg shells is uncommon at the time of oviposition, avian faecal material and other environmental sources in the laying house can rapidly introduce bacteria onto eggs (Board and Fuller, 1994). Inadequate sanitation in egg processing facilities is another possible cause of shell contamination (Davies and Breslin, 2003a). If not removed during processing, pathogens on the shell surface can be transferred to the edible, liquid portion of the egg, when the shell is broken to release the contents for use or consumption. Moreover, bacteria can also penetrate through shells to reach the contents. The porous shell is not a significant obstacle to bacterial penetration, although the underlying shell membranes are a more effective barrier (Burley and Vadehra, 1989; Ricke *et al.*, 2001). Eggs are routinely washed in some countries to remove pathogens and spoilage organisms from shells, but improper control of temperature during egg washing can lead to a pressure gradient that promotes the movement of microbes through the shell membranes and into the contents (Stadelman and Cotterill, 1995).

Diverse *Salmonella* serotypes are found on shells, but only SE has been associated with a large number of egg-transmitted, human disease outbreaks in recent decades. A Japanese study reported that SE was the only one of six serotypes tested that was deposited in egg yolks by experimentally-infected hens (Okamura *et al.*, 2001a). Similarly, a study in the UK found SE only inside naturally-contaminated eggs, even though a wide assortment of serotypes was present on the shells of these eggs (Humphrey *et al.*, 1991b). This suggests that some mechanism other than shell contamination is responsible for the current public health problems related to eggs. This other process, often referred to somewhat misleadingly as 'transovarian transmission', is the consequence of systemic infection of laying hens with *Salmonella* that results in deposition of

the pathogen inside the contents of developing eggs in the reproductive tract (Timoney *et al.*, 1989; Gast and Beard, 1990a).

1.2.2 Systemic infection of hens and transovarian transmission of *Salmonella* Enteritidis

Like most other paratyphoid (non-host-adapted) *Salmonella* serotypes, SE is usually introduced to chickens via the gastrointestinal tract. After oral ingestion from the environment, SE colonizes several regions of the tract, particularly the crop and caeca (Turnbull and Snoeyenbos, 1974). Invasion through mucosal epithelial cells can then lead to systemic dissemination to a wide array of internal organs, including reproductive tissues (Gast and Beard, 1990b; Humphrey *et al.*, 1993). By colonizing the ovary (the site of yolk maturation and release) and the oviduct (the site of albumen secretion around the descending yolk), SE appears to gain access to the contents of eggs (Miyamoto *et al.*, 1997; Okamura *et al.*, 2001a; De Buck *et al.*, 2004). Some investigators have found SE inside pre-ovulatory follicles and in developing eggs removed from the oviducts of infected hens before oviposition (Thiagarajan *et al.*, 1994; Keller *et al.*, 1995). Recent reports have also implicated *S.* Heidelberg as an egg-transmitted pathogen (Hennessy *et al.*, 2004), and an experimental-infection study documented the ability of some strains of this serotype to colonize reproductive tissues and be deposited inside eggs (Gast *et al.*, 2004).

In experimental infection studies, laying hens have typically produced internally-contaminated eggs for only a few weeks following oral inoculation (Gast and Beard, 1990a; Gast and Holt, 2000a). However, in commercial laying flocks, the patterns of egg contamination over time are far more irregular, as infection spreads gradually through each house. Contamination of eggs with SE seems to be a generally infrequent phenomenon within infected flocks. Two studies of environmentally-positive, commercial laying flocks in the USA have indicated a prevalence of contaminated eggs of less than 0.03% (Kinde *et al.*, 1996; Henzler *et al.*, 1998). The overall incidence of SE contamination of eggs from commercial flocks in the USA has been estimated at around 0.005% (Ebel and Schlosser, 2000). Likewise, egg contamination usually occurs at relatively low frequencies in experimental infection studies, even after the administration of very large oral doses of SE to laying hens (Humphrey *et al.*, 1991a; Gast and Holt, 2001a; Gast *et al.*, 2002).

1.2.3 Sources of introduction of *Salmonella* Enteritidis into poultry flocks

A recent national survey in the USA indicated that approximately 7% of the commercial laying flocks in that country were environmentally positive for SE (Garber *et al.*, 2003). The leading potential sources that can introduce SE into laying flocks are the replacement chicks themselves, the poultry house environment, rodents and other pests, and feed. Hatcheries, too, are significant because of the combined circumstances of possible vertical transmission of SE

from infected breeder flocks (Methner *et al.*, 1995; Berchieri *et al.*, 2001), the especially high susceptibility of newly hatched chicks to bacterial colonization of the intestinal tract (Duchet-Suchaux *et al.*, 1995; Gast and Benson, 1996), and the extensive circulation of contaminated dust and aerosols in the crowded conditions within hatcher cabinets (Davies *et al.*, 2001; Mitchell *et al.*, 2002).

Even if not exposed to SE as chicks or growing pullets, laying hens can still be infected subsequently with the pathogen, if transferred into a laying house that was not adequately decontaminated after removal of a previous, infected flock. A Dutch study (van de Giessen *et al.*, 1994) reported that most commercial flocks became infected for the first time as a result of environmental exposure to contaminated laying houses. A large field study in the USA (Schlosser *et al.*, 1999) showed that the presence of SE in laying house environments was strongly correlated with the probability that flocks would produce contaminated eggs. Environmental reservoirs of SE have sometimes been found to persist in laying houses, even after intensive cleaning and disinfection is applied upon termination of a flock (Davies and Wray, 1996; Davies and Breslin, 2003c). In one study, SE could still be isolated from litter, dried faeces and feed for 26 months after removal of the chickens (Davies and Breslin, 2003d). Even after effective cleaning and disinfection, pests such as mice can re-introduce SE into poultry farms (Davies and Wray, 1995a).

An extremely diverse assortment of vectors, including insects, reptiles, wild birds, rodents, livestock, pets and humans, can all transmit SE to poultry, their housing environment, or their feed and water sources. Insects, particularly beetles (Gray *et al.*, 1999) and flies (Olsen and Hammack, 2000), are common in poultry houses and can carry SE, both externally and internally. Mice have been the most consistent and convincing documented source of SE for contaminating poultry facilities. Environmental contamination with SE has often correlated directly with heavy mouse infestations (Henzler and Opitz, 1992; Schlosser *et al.*, 1999). Mice captured on poultry farms have been infected with SE at high frequencies and the droppings have been shown to be capable of transmitting the organism to chickens (Davies and Wray, 1995b; Guard-Petter *et al.*, 1997). Moreover, the use of molecular finger-printing has linked clones of SE found in mice, laying hens and eggs (Liebana *et al.*, 2003).

Feed is always a possible source of *Salmonella*, because of both the presence of the organisms in feed ingredients and the occurrence of reservoirs of contamination in feed mills (Davies and Wray, 1997; Whyte *et al.*, 2003; Jones and Richardson, 2004). However, actual epidemiological links between poultry feedstuffs and SE infections in either laying flocks or humans have been very infrequent (Poppe *et al.*, 1991; Veldman *et al.*, 1995). Nevertheless, in a Japanese study, serological and molecular typing connected isolates from feed and egg contents (Shirota *et al.*, 2001).

Once SE is introduced into a poultry house, environmental and management conditions can promote further distribution of the pathogen throughout the flock. In particular, airborne circulation of contaminated dust particles and aerosols can disseminate bacteria very widely (Nakamura *et al.*, 1997; Gast *et al.*, 1998;

Holt *et al.*, 1998). Reduction of circulating, airborne particulates by an electrostatic space-charge (negative air ionization) system has been reported to reduce the transmission of *Salmonella* infection to chicks under experimental conditions (Gast *et al.*, 1999; Mitchell *et al.*, 2002). Insect and rodent vectors, human activity and poultry house equipment can also transport bacterial pathogens within laying flocks.

1.2.4 Host and bacterial factors that promote *Salmonella* Enteritidis infection in poultry and egg contamination

Differences in the susceptibility of chickens to SE infection can lead to corresponding differences in the likelihood that contaminated eggs will be produced. One parameter that has considerable influence on the susceptibility of chickens to *Salmonella* is their age. Newly hatched chicks lack a complete gastrointestinal microflora to serve as a protective barrier against colonization by pathogens (Stavric *et al.*, 1987) and, accordingly, are highly susceptible to infection. Large oral doses of SE can be lethal for one-day-old chicks (Gast and Benson, 1995), but mortality is much less common when chicks are infected at one week of age or more (Duchet-Suchaux *et al.*, 1995). Infection of very young poultry can also lead to highly persistent intestinal colonization. After experimental exposure of chicks to SE during the first few days of life, the pathogen can persist in the intestinal tracts of many birds for six months or more (Phillips and Opitz, 1995; Gast and Holt, 1998a).

Another issue with significance for the outcome of SE infections concerns the role of genetically-based differences in susceptibility between various lines of chickens. These lines have been reported to differ in the observed frequencies of mortality, organ invasion and egg contamination, following SE inoculation of the live birds (Beaumont *et al.*, 1994; Protais *et al.*, 1996). Differences between lines have also been observed in resistance to persistent intestinal colonization by SE (Beaumont *et al.*, 1999; Berchieri *et al.*, 2001). However, the mechanisms that are responsible for these genetic differences in susceptibility remain incompletely characterized.

A poultry management practice that affects host susceptibility to SE is the use of induced molting by feed deprivation to extend the productive lives of commercial egg-laying flocks. Feed deprivation has been found to increase faecal shedding of SE (Holt and Porter, 1992) and invasion of internal organs (Holt *et al.*, 1995) in orally inoculated hens. Moreover, induced molting can reduce the oral dose of SE needed to establish intestinal colonization (Holt, 1993) and increase the frequency of horizontal transmission between hens (Holt, 1995). Inducing molting by feeding low-nutrient-density substances, such as wheat middlings, has been shown to have significantly less effect on the course of SE infections than does feed deprivation (Seo *et al.*, 2001).

Several bacterial attributes appear to be relevant to determining whether SE will be deposited in eggs laid by infected chickens. The ability to cause egg contamination in experimentally-infected hens has been shown to vary among

SE strains (Gast and Holt, 2000a, 2001c). The expression of potential virulence factors, including flagella, fimbriae, outer membrane proteins and iron-uptake systems, can be influenced by pH and temperature conditions, or by growth in chicken tissues (Chart *et al.*, 1993; McDermid *et al.*, 1996; Walker *et al.*, 1999). Serial *in vivo* passage of an SE isolate through reproductive tissues of groups of laying hens has led to an increase in its frequency of deposition in eggs (Gast *et al.*, 2003). Phenotypic properties, such as growth to high cell densities and the expression of high molecular mass lipopolysaccharides have also been linked to egg contamination (Guard-Petter, 1998, 2001). Multiple microbial attributes, such as the abilities to invade beyond the intestinal tract and to colonize reproductive tissues, may complement each other to produce egg contamination (Guard-Petter, 2001; Gast *et al.*, 2002).

1.3 Characteristics of *Salmonella* contamination of eggs

1.3.1 Deposition of *Salmonella* Enteritidis in eggs: quantity and location

Naturally-contaminated eggs have usually been found to harbor very small numbers of SE cells when tested at short intervals following oviposition. Typically, fewer than ten SE cells are present in each contaminated egg (Humphrey *et al.*, 1989), although much larger bacterial populations have been observed in a small proportion of eggs (Humphrey *et al.*, 1991b). Even after the administration of extremely high oral doses of SE (sometimes as many as 10^9 cells) to hens in experimental infection studies, relatively small numbers of contaminants are generally found in the contents of freshly laid eggs (Gast and Beard, 1992). In one such study, most of the eggs from inoculated hens contained less than one SE cell per ml of liquid egg contents, and none contained more than 67 cells per ml (Gast and Holt, 2000a).

Experimentally infected hens have been reported to deposit SE in either (or sometimes both) the yolk or albumen of developing eggs (Humphrey *et al.*, 1989, 1991b; Gast and Beard, 1990a; Bichler *et al.*, 1996; Gast and Holt, 2000a), perhaps as a consequence of the colonization of different regions of the reproductive tract (ovary or oviduct). Intensive microbiological examination of the yolks of eggs laid by experimentally inoculated hens has indicated that SE is deposited far more frequently in association with the vitelline membrane than inside the yolk contents (Gast and Beard, 1990a; Gast and Holt, 2001a). The predominant perspective on naturally occurring contamination of eggs is that SE is initially deposited more often in the albumen (or at least outside the vitelline membrane) than in the yolk (Humphrey, 1994). This point of view is supported by the relatively small numbers of bacteria that are normally detected inside fresh eggs, because more rapid microbial multiplication to higher numbers would be expected in the nutrient-rich yolk than in the growth-restricting conditions of the albumen. Risk assessment efforts in the USA, conducted to provide an analytical foundation for the development of regulatory responses to control the transmission of SE by eggs, have been built around the assumption

that egg contamination is most often initiated by deposition of the pathogen in the albumen or on the vitelline membrane (Hope *et al.*, 2002; Latimer *et al.*, 2002).

1.3.2 Survival and multiplication of *Salmonella* Enteritidis in albumen and yolk

The avian egg has numerous physical and biochemical barriers to microbial growth that are intended to protect the developing embryo from exposure to pathogens (Burley and Vadehra, 1989; Board and Fuller, 1994; Stadelman and Cotterill, 1995). Although the egg shell itself is rather porous, it is coated with a proteinaceous cuticle and has two underlying shell membranes to provide additional resistance to bacterial penetration. Nevertheless, *Salmonella* and other bacteria are able to move through the external structures of the egg, especially at the large end, where the shell membranes separate to form an air cell (Berrang *et al.*, 1999). The creation of negative pressure inside eggs, when the contents contract during cooling, and the presence of moisture and faecal matter on the shell can promote bacterial penetration into eggs (Berrang *et al.*, 1999). Inside the egg, several components of the albumen are directly or indirectly antimicrobial (Ricke *et al.*, 2001; see also Table 1.1). The most significant of these antibacterial albumen proteins is ovotransferrin, which binds iron to limit its availability to support microbial growth (Baron *et al.*, 1997). Furthermore, the pH of albumen increases as it ages and thereby becomes more inhibitory to bacterial multiplication (see Table 1.2).

Despite this considerable array of protective constituents, SE is able to survive and sometimes even grow slowly in albumen. Several investigators have reported that SE inoculated into separated albumen was able to persist during incubation at warm temperatures for days or even weeks (Lock and Board, 1992; Gast and Holt, 2000b, 2001b), although a decline in the numbers of SE cells in albumen has been noted during refrigerated storage (Stephenson *et al.*, 1991). After inoculation into the albumen of whole eggs, at sites remote from the yolk,

Table 1.1 Principal antibacterial proteins in albumen of chicken eggs

Protein	Proportion of total protein (%)	Antibacterial properties
Ovotransferrin	12	Binds iron and other metal ions
Ovomucoid	11	Inhibits activity of trypsin
Lysozyme	3.4	Causes lysis by hydrolyzing β-1,4 glycosidic bonds in cell walls
Ovoinhibitor	1.5	Inhibits several proteases
Ovoflavoprotein	0.8	Binds riboflavin
Avidin	0.06	Binds biotin

Sources: Derived from information in Burley and Vadehra (1989), Board and Fuller (1994), and Stadelman and Cotterill (1995).

Table 1.2 Changes in the contents of chicken eggs during storage

Component	In eggs at oviposition	In eggs after storage
Air cell	Small	Larger, due to loss of water and CO_2 from albumen
Albumen	Firm, holds yolk in center; pH ~ 7.6	Thinner, more fluid and less gelatinous, because of water and CO_2 loss; pH ~ 9.5
Yolk	Denser than albumen	Enlarged and less dense, due to water uptake from albumen following degradation of vitelline membrane integrity

Sources: Adapted from information in Burley and Vadehra (1989), Board and Fuller (1994), and Stadelman and Cotterill (1995).

a modest degree of growth has sometimes been observed after several days of incubation at 20 °C or higher (Gast and Holt, 2000b; Cogan *et al.*, 2001). Multiplication of SE proceeds faster in fresh than in stored albumen, possibly due to an increase in pH during storage (Messens *et al.*, 2004). Perhaps by inactivating ovotransferrin and other antibacterial proteins, pasteurization has been found to render albumen less resistant to bacterial growth (Baron *et al.*, 1999).

In egg yolk, nutrients are present in abundance and the antimicrobial albumen proteins are absent, so the growth of SE can be rapid and prolific (Clay and Board, 1991). Even very small initial numbers of SE cells can multiply to reach dangerously high concentrations within a single day, after inoculation into egg yolk (Gast and Holt, 2000b). Temperature is the principal factor that affects SE growth in egg yolks. Extensive multiplication has been reported at 15 °C and higher, whereas slower multiplication is evident at 10 °C and growth ceases at around 4 °C (Kim *et al.*, 1989; Saeed and Koons, 1993; Schoeni *et al.*, 1995; Gast and Holt, 2000b).

Even if SE is not located initially inside the yolk contents of contaminated eggs, but, instead, is deposited on the exterior surface of the vitelline membrane or in nearby areas of the albumen, bacterial penetration through the membrane could still result in extensive multiplication within yolks. Using various *in vitro* models for egg contamination, the penetration of SE through the yolk membrane has been reported to occur at a wide range of frequencies (Hammack *et al.*, 1993; Humphrey and Whitehead, 1993; Braun and Fehlhaber, 1995; Gast and Holt, 2000b). However, in a similar study, no movement of *Salmonella* from the exterior to the interior of the yolk membrane was observed (Fleischman *et al.*, 2003). The migration of SE across the vitelline membrane into the yolk has been shown to increase with the level of contamination, storage temperature and egg age (Braun and Fehlhaber, 1995; Gast and Holt, 2000b).

Another mechanism by which SE could eventually begin to multiply rapidly after deposition in the albumen involves the gradual degradation of the vitelline membrane, leading to the release of yolk nutrients into the albumen, as the egg

ages (Humphrey, 1994; see also Table 1.2). This deterioration of the yolk membrane is accelerated by abusively high temperature conditions (Hara-Kudo *et al.*, 2001; Latimer *et al.*, 2002). In experimentally contaminated eggs, the growth of SE in areas of the albumen around the yolk increased with the age of the eggs at inoculation (Humphrey and Whitehead, 1993). However, rapid growth of SE in albumen, due to yolk-membrane degradation, has been observed after only three weeks of storage at 20 °C (Humphrey and Whitehead, 1993).

1.3.3 Implications for detecting *Salmonella* Enteritidis in eggs

The nature of SE deposition in eggs has a profound effect on the methods that have evolved for detecting contamination. Because SE deposition is evidently a highly infrequent event and, because contaminated eggs have usually been found to contain very low concentrations of SE cells, large numbers of eggs must be sampled to ensure that the pathogen is detected with adequate sensitivity (Gast, 1993). This imposes several significant constraints on practical and dependable methods for detecting the organism in eggs taken from commercial laying flocks. To sample large numbers of eggs without overwhelming available laboratory resources, the contents of up to 20 eggs are often pooled together. However, pooling eggs introduces a dilution of the already small numbers of SE cells. Accordingly, incubation of egg pools, before applying subsequent enrichment culture steps, is essential to permit the multiplication of SE to more consistently detectable levels (Gast, 1993; Gast and Holt, 2003). Supplementing these pools with concentrated sources of iron and other nutrients can improve the growth rate of SE in incubating egg content pools (Gast and Holt, 1998b; Chen *et al.*, 2001). Innovative rapid technologies for detecting SE can be applied to eggs to replace traditional culture methods, but are still dependent on a preliminary egg-pool incubation step to achieve satisfactory detection sensitivity (Gast and Holt, 2003).

1.3.4 Implications for refrigeration or pasteurization of eggs

The nature of SE deposition in eggs also has significant consequences for the application of refrigeration or pasteurization as measures to protect consumers from egg-transmitted illness. Refrigeration of eggs at 7 °C during storage and transportation has been recommended repeatedly for preventing the multiplication of small initial numbers of SE cells to more dangerous levels (US Department of Agriculture, 1998; US Food and Drug Administration, 2004). However, refrigeration of eggs using conventional technologies may require several days before temperatures within the eggs are reduced sufficiently to prevent further microbial growth (Curtis *et al.*, 1995; Thompson *et al.*, 2000). If SE is deposited in the albumen, where bacterial multiplication is very slow, even at warm temperatures, the extended interval necessary to achieve growth-restricting temperatures inside eggs will have little adverse effect. However, if the initial site of SE contamination is in or on the nutrient-rich yolk, rapid

multiplication could produce high levels of the pathogen during the early stages of refrigerated storage, while internal egg temperatures are still in the process of being reduced. Determining how often SE is in fact deposited in association with the yolk, and whether (and how quickly) it can penetrate through the vitelline membrane into the yolk, is, accordingly, very important in defining the necessary parameters for thoroughly protective application of egg refrigeration. Many of these same considerations affect the ultimate efficacy of egg pasteurization, as the number of bacteria that will survive destruction by heat (either inside intact shell eggs or in liquid egg products) under any specific combination of time and temperature will depend on the numbers of cells that were present initially (Hou *et al.*, 1996; Brackett *et al.*, 2001). Therefore, the effect of the location of deposition on subsequent growth to high numbers before pasteurization becomes a pivotal consideration in this context as well. Techniques for reducing pathogens in eggs are discussed in more detail in Chapter 12.

1.4 Future trends

Considerable public and private resources have been invested throughout the world in attempting to control the egg-borne transmission of SE. A risk assessment study performed in the USA recommended intervention at multiple steps in the farm-to-fork continuum, as the most productive overall strategy (Hope *et al.*, 2002). As already discussed in the previous section, refrigerated storage and pasteurization of eggs are highly effective post-production options for risk mitigation. Nevertheless, the preponderance of effort and expenditure has been devoted to controlling SE infections in laying flocks. In the early years after SE was first identified as a significant public health problem, most control plans focused on trace-back testing and eradication efforts. For example, in a national programme that was instituted in the USA from 1990 to 1995, flocks were tested after being implicated as the sources of eggs that had caused human disease outbreaks. This plan mandated either the diversion of eggs for pasteurization or depopulation of the laying house, when the flock was found to be infected (US Department of Agriculture, 1991; Hogue *et al.*, 1997b). During the term of this control programme, restrictions were imposed on 31 laying flocks, resulting in the voluntary depopulation of nearly nine million laying hens and the diversion of more than one billion eggs for pasteurization. However, during this same period of time, the overall incidence of SE in both poultry and eggs in the USA continued to increase (Hogue *et al.*, 1997a). The apparent failure of this entirely reactive trace-back approach illustrates the inherent impossibility of identifying and eradicating all infected flocks in the face of continuous re-introduction of SE into laying flocks from diverse environmental sources.

In recent years, an assortment of microbial quality-assurance programmes for commercial laying flocks have been proposed and implemented by government

agencies and by the poultry industry (Hogue *et al.*, 1997b; US Food and Drug Administration, 2004). These programmes have represented a more proactive, and thereby far more effective, alternative to trace-back eradication. Most of these programmes combine a battery of risk-reduction practices for egg producers, with a testing component designed to identify problem flocks for further attention (sometimes including regulatory intervention). The testing part of these programmes also serves as a means of assessing the ongoing efficacy of the risk-reduction practices to ensure that the commitment of resources to quality assurance programmes is cost-effective. In the most common approach to testing, environmental samples are collected and tested to screen for flock infection, and egg samples are subsequently tested to determine whether an ongoing threat to public health exists. Eggs from flocks that test positive must generally be diverted for pasteurization (Hogue *et al.*, 1997b; US Food and Drug Administration, 2004). Risk-reduction practices that are common to most quality assurance schemes include using chicks from flocks that are certified as uninfected by breeder-flock testing protocols, such as those of the National Poultry Improvement Plan in the USA (Rhorer, 1999), implementing effective procedures for controlling rodents and other pests, heightened biosecurity measures for poultry facilities, thorough cleaning and disinfection of facilities between flocks and refrigeration of eggs as soon as possible after collection. This type of approach has been associated with significant reductions in the incidence of SE infections in both egg-laying flocks and humans in several states in the USA (White *et al.*, 1997; Mumma *et al.*, 2004).

Another important tool for combatting SE infection in poultry is vaccination. Vaccination of pullets or hens with either killed or live preparations has reduced (but not entirely prevented) faecal shedding, organ invasion and egg contamination, following challenge with SE (Gast *et al.*, 1992, 1993; Zhang-Barber *et al.*, 1999). This protection can be particularly significant for highly susceptible hens undergoing an induced molt (Holt *et al.*, 2003; Nakamura *et al.*, 2004). However, vaccination does not construct an impenetrable barrier to SE infection, since protective immunity induced by vaccines has been overcome occasionally by high challenge doses. A field study in the USA found no significant protective effect against SE that could be attributed to vaccination of commercial laying flocks (Davison *et al.*, 1999). Poor vaccine performance has sometimes been tied to severe rodent or sanitation problems in laying houses (Davies and Breslin, 2003b). Nevertheless, even when vaccination has not completely prevented SE infection in commercial flocks, it has generally been able to accomplish meaningful reductions in egg contamination (Davies and Breslin, 2004). In the UK, a declining prevalence of SE infections in humans was observed to follow the initiation of widespread vaccination of laying hens (Cogan and Humphrey, 2003). Vaccination may be most valuable as an adjunct to other risk-reduction practices, especially when applied to highly susceptible flocks or flocks exposed to severe challenges from environmental sources.

The most promising option for achieving sustainable reductions in the prevalence of contaminated eggs appears to be the patient and persistent

application of risk-reduction programmes of verified efficacy. However, one potential area of vulnerability in microbial quality-assurance schemes for shell eggs is created by the possibility that *Salmonella* serotypes other than SE might become significant sources of egg-transmitted human disease. Although the epidemiological association between SE and eggs has been strong and unique, other paratyphoid serotypes (including *S.* Typhimurium, *S.* Heidelberg and *S.* Thompson) have also been reported to be capable of colonizing reproductive organs of chickens and thereby causing egg contamination (Snoeyenbos *et al.*, 1969; Cox *et al.*, 1973; Keller *et al.*, 1997; Okamura *et al.*, 2001b; Gast *et al.*, 2004). Recently, the Centers for Disease Control and Prevention in the USA have implicated eggs and egg-containing foods as the principal sources of human *S.* Heidelberg infections (Hennessy *et al.*, 2004). Nevertheless, several pivotal aspects of current risk-reduction efforts, such as testing and vaccination, focus almost exclusively on identifying or controlling SE and are not intended to address the possible presence of other *Salmonella* serotypes in eggs. Although targeting control measures to specific disease agents is crucial for mounting rapid responses to public health emergencies, risk-reduction practices that are not inherently agent-specific (such as biosecurity, rodent control, cleaning and disinfection and egg refrigeration) may be of even greater long-term importance because of their ability to minimize the opportunities for another pathogen to emerge and cause a new egg-borne disease crisis.

1.5 Sources of further information and advice

The most comprehensive, single source of information about SE in eggs and chickens is Saeed *et al.* (1999). This book contains 39 chapters relating to the subject, subdivided into sections on international public health issues, molecular epidemiology, virulence and pathogenesis, and prevention and control. General texts that provide extensive background information about eggs (including microbiological considerations) are Burley and Vadehra (1989), Board and Fuller (1994) and Stadelman and Cotterill (1995). Despite having been written ten years ago, a review of egg contamination problems by Humphrey (1994) remains very useful in its treatment of the principal issues. The most thorough description of avian *Salmonella* infections is found in Gast (2003). Although it covers other domestic animals in addition to poultry, several chapters in Wray and Wray (2000) provide good coverage of central themes relating to *Salmonella* in chickens. Guard-Petter (2001) offers a thought-provoking review of the mechanisms by which SE causes egg contamination. The epidemiology of human SE infections in the USA is addressed by Hogue *et al.* (1997b) and Patrick *et al.* (2004). The government-sponsored risk assessment for SE in eggs in the USA is described by Hope *et al.* (2002). The record of the effectiveness of egg-quality assurance programmes in influencing the epidemiology of SE in the USA is documented by Mumma *et al.* (2004). Considerable information about both the SE problem and responses to it by public health and regulatory agencies

can be found in the corresponding official websites (examples are www.cdc.gov, www.fda.gov, and www.fsis.usda.gov in the USA). A particularly good presentation of a state egg-quality assurance scheme is found at http://ulisse.cas.psu.edu/ext/Comeggs.html.

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